which analysed and assessed the oil and found a purchaser for it, thus resolving one of the major problems in establishing a new oil. Further developments in the techniques of growing ninde and distilling the oil have been made subsequently.

The major constituents of ninde oil have been isolated by column chromatography and gc and identified from mass spectra and IR data. Similar studies have been made on Indian and Brazilian palmarosa oil, as shown in Table 1.

TABLE 1. ESSENTIAL OILS CONSTITUENTS OF NINDE AND PALMAROSA

Constituent	Ninde	Indian palmarosa	Brazilian palmarosa	Constituent	Ninde	Indian palmarosa	Brazilian palmarosa
Myrcene	0·02	0·07	0·13	Neral	0·48	0·24	0·11
Limonene	T	0·26	0·06	Geranyl formate	0·34	—	—
Ocimene	0·07	0·11	0·28	Geranial	2·21	6·85	0·24
γ-Terpinene	—	0·35	1·08	Geranyl acetate	4·54		6·96
Methyl Heptenone	—	T	0·03	Nerol	0·55	0·33	0·30
Linalool	1·71	0·59	2·58	Geraniol	87·61	88·37	86·16
Caryophyllene	—	0·35	0·93	Caryophyllene oxide	T	T	0·14
α-Guaiene	1·23	—	—	Farnesol	—	0·07	0·17

T = Trace.

The resemblances between ninde oil and the two types of palmarosa are very striking, with constituents making up more than 95% of the oils being common to all.

Factors Influencing Production of Patchouli Sesquiterpenes in Cultured Cells and Regenerated Plantlets

L. H. JONES and P. P. S. KRISHNADETHAN

Unilever Research Laboratory Colworth/Welwyn, Sharnbrook, Bedfordshire

PATCHOULI oil is a complex mixture of sesquiterpenes obtained from the leaves of *Pogostemon cablin* Benth. The major components are patchouli alcohol, α-, β- and γ-patchoulene, α-guiaene and α-bulnescene. The oil is secreted in a variety of specialized glandular cells, both on the leaf surface, and within the leaf. The external glands (glandular trichomes) are similar to those of mint, but the internal glands appear to be a unique feature of the plant.[1] In both cases the glandular cells are closely associated with the photosynthetic cells of the leaf, although some internal glands also occur in stem and even root tissues. In these cases glands are located in the phloem.

Our problem was to grow patchouli cells in nutrient culture and attempt to induce patchouli oil synthesis. Although vigorous cultures were obtained, no success was obtained in detecting even minute traces of patchouli sesquiterpenes. However, patchouli cells in culture readily regenerated plantlets, and glandular trichomes were present from the earliest signs of organization. Analysis of gland contents showed only a series *n*-alkanes and no terpenoid hydrocarbons to be present although the glands appeared to possess normal morphology.[2]

Cultivated patchouli does not normally flower, and the crop is always vegetatively propagated. Volkhovr skaya[3] reported the induction of flowers in 8 hr days but no seed was set. We therefore do not know whethe-

[1] HENDERSON, W., HART, J. W., HOW, P. and JUDGE, F. J. (1970) *Phytochemistry* **9**, 1219.
[2] HART, J. W., WOODCOCK, G. J. and WILSON, L. (1970) *Ann. Bot.* **34**, 137, 789.
[3] VOLKHOVSKAYA, U. V. (1968) *Tr. Sukhum Opyt. Sta. Efirnomaslich. Kult.* **7**, 33.

juvenile seedling stages exist when patchouli oil biosynthesis is absent. Once switched on, synthesis apparently continues and oil bearing glands are present in large numbers on the second leaf primordium in the normal vegetative apex. Hence the youngest available cuttings always contain oil.

Plants derived from cultured material were grown on in growth rooms but possessed abnormal leaf morphology and did not accumulate patchouli sesquiterpenes. Two changes were made in the growth conditions earlier used.[1] Glucose was changed to sucrose in the medium and immediately there was good rooting, which had hitherto been poor, and normal leaf morphology Perfectly normal looking patchouli plants were obtained, but no sesquiterpenes appeared in the glands. These were allowed to grow in a glasshouse, where at that particular time of year they experienced long days. A full spectrum of patchouli oil sesquiterpenes was produced This immediately raised the question of whether daylength and/or light quality was an important regulation of sesquiterpene synthesis. In *Mentha*, long days and low night temperatures are required.[4,5] Our experiments indicated that both daylength and light quality may be important.

In one experiment regenerated plants were grown in 12 and 18 hr days at two light intensities with day temp 20° and night temp. 15°. Sesquiterpenes developed faster in long days than in short days, patchouli alcohol being the last major peak to appear. The time of first appearance of sesquiterpenes was not affected by light intensity. In order to test whether the daylength effect was phytochrome controlled, plantlets were grown in short days with red (R), and R followed by far-red (FR) night breaks No sesquiterpenes developed in either regime until the night temp. was lowered, when they appeared in both treatments. The system is therefore not phytochrome controlled.

The importance of low night temperature and long days suggest that reduction of respiratory drain is an important factor. In the light, acetyl CoA could be available for terpene synthesis, whereas in the dark it would be utilized in the Krebs' cycle. The close association of glandular trichomes with photosynthetic palisade cells of the leaf suggest rapid conversion of photosynthate into oil, which may subsequently be used as a respiratory substrate. Such a mechanism may be inferred from the results of Francis.[6]

From the complexity of the specialized structures involved and the interdependence with environment it seems unlikely that cultured patchouli cells will be induced to produce the sesquiterpenes typical of patchouli oil. However, the free regeneration of plantlets from culture could be utilized in conjunction with mutagenic treatments followed by selection for improved oil production in a crop, which being non flowering has little inherent variability.

[4] STEWARD, F. C., HOWE, K. J., CRANE, F. A. and RALESON, R R. (1962) *Cornell Univ Agr. Expt. Sta. Mem.* 379.
[5] LANGSTON, R. and LEOPOLD, A. C. (1954) *Proc. Am. Soc. Hort. Sci.* **63**, 347
[6] FRANCIS, M. J O. (1972) *Planta Med* **22**(2), 201.

Instrumental Techniques for the Analysis of Essential Oils

P. TEISSEIRE

Centre de Recherche, Société Roure-Bertrand-Dupont, Grasse, France

THE existence in an essential oil of components in very small amounts characterizes the organoleptic properties of that essential oil. Therefore, an intimate knowledge of these components is indispensable when it is desired to obtain a fully satisfactory artificial reproduction of the essential oil.

Such a study requires highly advanced instrumental techniques. In the present paper, I shall describe the chief techniques used. The primary object is to collect micro-samples of compounds and then to determine their various spectral properties for the purpose either of identifying them with known products, or of characterizing them. A detailed technological study of the instrumental means used in micropreparative GLC is described. The technology of the quantitative transfer of the micro-samples separated above to the various instruments used in spectrometry (IR, NMR) is given. Examples of the application of the above techniques to the study of the minor components of some essential oils follow.

Oil of Cedarwood Atlas (*Cedrus Atlantica*). Characterization of γ-himalachene, of dehydro-aryl-himalachene and of α-epoxy-6,7-himalachene.

Oil of Ylang-Ylang. Identification of copaene, caryophyllene, D-germacrene and α-farnesene We show, also, that the presence of all these four sesquiterpenic hydrocarbons in Oil of Basil from the Comoro Islands, Reunion or Madagascar provides proof of the adulteration of Oil of Basil with Oil of Ylang-Ylang.

Oil of Patchouli. (a) *Sesquiterpenic hydrocarbons*. 13 hydrocarbons were isolated, 8 were definitely identified: these are β-patchoulene, α-patchoulene, seychellene, α-bulnesene, α-guaiene, caryophyllene, β-elemene and α-humulene. (b) *Sesquiterpenic epoxides*. Epoxy-1α,5α-guaiene 4, epoxy-1,10-α-bulnesene 5 and epoxy-caryophyllene. (c) *Ethylenic alcohols and keto-alcohols*. Besides patchoulol and pogostol, there are at least 2 other ethylenic alcohols and 3 ethylenic keto-alcohols.